

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding a *cis*-prenyltransferase enzyme, selected from the group consisting of:
 - 5 a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NOs:4 and 6;
 - b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS
10 followed by 0.1X SSC, 0.1% SDS; or
an isolated nucleic acid molecule that is complementary to (a) or (b).
2. An isolated nucleic acid molecule as set forth in SEQ ID NOs: 3 and 5.
3. A polypeptide encoded by the isolated nucleic acid molecule of
15 Claim 1.
4. A polypeptide encoded by the isolated nucleic acid molecule of Claim 2.
5. A polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:6.
- 20 6. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 301 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:4 or a second nucleotide sequence comprising the complement of
25 the first nucleotide sequence, wherein said enzyme has *cis*-prenyltransferase activity.
7. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 168 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment
30 when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6 or a second nucleotide sequence comprising the complement of the first nucleotide sequence, wherein said enzyme has *cis*-prenyltransferase activity.
8. A chimeric gene comprising the isolated nucleic acid molecule
35 of Claim 1 operably linked to suitable regulatory sequences.
9. A transformed host cell comprising the chimeric gene of Claim 8.

10. The transformed host cell of Claim 9 wherein the host cell is selected from the group consisting of plant cells and microbial cells.

11. A host cell according to Claim 10 selected from the group consisting of russian dandelion (*Taraxacum kok-saghyz*), rubber tree
5 (*Hevea brasiliensis*), guayule (*Parthenium argentatum*), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.), tomato (*Lycopersicon* spp.), potato (*Solanum* spp.), hemp (*Cannabis* spp.), sorghum (*Sorghum vulgare*), wheat (*Triticum* spp.), maize (*Zea mays*), rice (*Oryza sativa*), rye (*Secale cereale*), oats (*Avena* spp.), barley (*Hordeum vulgare*), rapeseed
10 (*Brassica* spp.), broad bean (*Vicia faba*), french bean (*Phaseolus vulgaris*), other bean species (*Vigna* spp.), lentil (*Lens culinaris*), soybean (*Glycine max*), arabidopsis (*Arabidopsis thaliana*), cotton (*Gossypium hirsutum*), petunia (*Petunia hybrida*), flax (*Linum usitatissimum*) and carrot (*Daucus carota sativa*).

12. The transformed host cell of Claim 10 wherein the host cell is selected from the group consisting of *Aspergillus*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Bacillus*, *Escherichia*, *Salmonella* and *Shigella*.

13. A method of obtaining a nucleic acid molecule encoding a *cis*-prenyltransferase enzyme comprising:
20 a) probing a genomic library with the nucleic acid molecule of Claim 1;
b) identifying a DNA clone that hybridizes with the nucleic acid molecule of Claim 1; and
c) sequencing the genomic fragment that comprises the
25 clone identified in step (b),

wherein the sequenced genomic fragment encodes a *cis*-prenyltransferase enzyme.

14. A method of obtaining a nucleic acid molecule encoding a *cis*-prenyltransferase enzyme comprising:
30 a) synthesizing at least one oligonucleotide primer corresponding to a portion of the sequence selected from the group consisting of SEQ ID NOs:3 and 5; and
b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a);

35 wherein the amplified insert encodes a portion of an amino acid sequence encoding a *cis*-prenyltransferase enzyme.

15. The product of the method of Claims 13 or 14.

16. A method of altering the level of expression of a plant *cis*-prenyltransferase protein in a host cell comprising:

- (a) transforming a host cell with the chimeric gene of Claim 8 and;
- 5 (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of altered levels of a plant *cis*-prenyltransferase protein in the transformed host cell relative to expression levels of an untransformed host cell.

17. A method according to Claim 16 wherein the method of altering the level of expression of a plant *cis*-prenyltransferase protein in a host cell comprises over-expressing at least one *cis*-prenyltransferase gene selected from the group consisting of SEQ ID NOs: 3 and 5.

18. A method according to Claim 16 wherein the method of altering the level of expression of a plant *cis*-prenyltransferase protein in a host cell comprises over-expressing the *cis*-prenyltransferase gene on a multicopy plasmid.

19. A method according to Claim 16 wherein said chimeric gene is operably linked to an inducible or regulated promoter.

20. A method according to Claim 16 wherein chimeric gene is expressed in antisense orientation.

21. A method according to Claim 16 wherein said chimeric gene is disrupted by insertion of foreign DNA into the coding region.

22. A method according to Claim 16 wherein the altering the level of expression of a plant *cis*-prenyltransferase protein results in a modulation in the defense mechanism of the plant.

23. A method for the production of natural rubber compounds comprising:

- 30 a) providing a transformed host cell comprising:
 - (i) suitable levels of isopentenyl pyrophosphate; and
 - (ii) a *cis*-prenyltransferase gene selected from the group consisting of SEQ ID NOs: 3 and 5, wherein said genes are operably linked to suitable regulatory
- 35 b) growing the transformed host cell of (a) under conditions whereby a natural rubber compound is produced.

24. A method for the identification of a polypeptide having *cis*-prenyltransferase activity in a rubber-producing plant comprising:

- (a) obtaining the amino acid sequence of a polypeptide suspected of having *cis*-prenyltransferase activity; and
 - 5 (b) aligning the amino acid sequence of step (a) with the amino acid sequence of a *cis*-prenyltransferase consensus sequence selected from the group consisting of SEQ ID NO:4, 6, 8, 9, and 10, wherein the alignment shows the presence of conserved domains I, IV, and V.
- 10 (SEQ ID NOs: 38-40).

25. A method for the identification of a polypeptide having *cis*-prenyltransferase activity in a rubber-producing plant comprising:

- (a) obtaining the amino acid sequence of a polypeptide suspected of having *cis*-prenyltransferase activity; and
 - 15 (b) aligning the amino acid sequence of step (a) with the amino acid sequence of a *cis*-prenyltransferase consensus sequence selected from the group consisting of SEQ ID NO:4, 6, 8, 9, and 10, wherein the alignment shows a sequence of at least about 50 non-conserved
- 20 amino acids present between the absolutely conserved tyrosine of Domain IV and the first of the absolutely conserved arginine residue of Domain V.